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Alteration in Electroencephalogram and Monoamine Concentrations in Rat Brain following Ibogaine Treatment

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ABSTRACT: Ibogaine (IBO) is a psychoactive indole alkaloid that has antiaddictive properties. However, treatment with IBO may lead to neurotoxicity, since IBO and its metabolites interact persistently with many neurotransmitter systems. Here, we recorded cortical electroencephalogram (EEG) signals from rats anesthetized with isoflurane. The heart rate (HR) was monitored via electrocardiogram (EKG) electrodes. After the baseline EEG was recorded, rats received one intraperitoneal (i.p.) dose of 50 mg/kg IBO. EEG signals were recorded for 2 hr. Rats were then sacrificed and brains dissected into frontal cortex (FC), caudate nucleus (CN), hippocampus (HIP), and brain stem (BS). The level of dopamine (DA), serotonin (5-HT), and their metabolites were determined by high-performance liquid chromatography with electrochemical detection (HPLC-ECD). Compared with baseline, a decrease in HR immediately after IBO injection and a decrease in δ , θ , α , and β power spectra frequency bands (1–4, 4–8, 8–13, 13–32 Hz) during the first 30 min after IBO administration was observed. EEG recovered within the next 15 min. In CN, the level of DA decreased and DA turnover rate increased significantly. The levels of 5-HT increased in FC. The pattern of EKG and EEG response to IBO may be due to multiple receptor interactions of IBO.

INTRODUCTION

Ibogaine is a psychoactive indole alkaloid derived from the roots of the West African rain forest shrub *Tabernanthe iboga*. Anecdotal reports and results gathered from animal studies have indicated that ibogaine may reduce craving for cocaine and is effective in decreasing the signs of opiate withdrawal.^{2,4} The worldwide social and medical problems of substance abuse make it of prime importance to evaluate the efficacy and side effects of this potentially useful antiaddictive compound.

The mechanism of ibogaine's claimed antiaddictive action is unclear. Ibogaine is known to interact with dopaminergic, serotonergic, cholinergic, muscarinic, opiate and *N*-methyl-D-aspartate (NMDA) receptor sites.^{15,5,16,12} It has been hypothesized that the therapeutic action of ibogaine may be linked to its NMDA receptor antagonist properties¹³ and/or persistent interactions with other neurotransmitter systems.⁹

Studies of O'Hearn and Molliver¹¹ and Scallet *et al.*¹⁴ have raised the question whether the mechanism of ibogaine's action that underlies its possible antiaddictive effects, may also lead to neurotoxicity. These investigators have shown that ibogaine in

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high doses induces necrotic damage to Purkinje neurons in the rat cerebellum. Although Molinari *et al.*¹⁰ did not confirm this observation in rats treated with lower doses of ibogaine, possible neurodegenerative effects of ibogaine remain a concern in the perspective of its future therapeutic use. The aim of our study was to assess the effects of acute ibogaine exposure in the rat using electrophysiological and neurochemical endpoints.

MATERIALS AND METHODS

Animals and Maintenance

Male adult Sprague-Dawley rats of Charles River cesarean delivered (CD) strain (Kingston, NY) obtained from the NCTR in-house breeding colony were used in this study. Animals were housed under controlled environmental conditions (temperature 22°C, relative humidity 45–55%, 12 hr light:dark cycle with lights off at 1800 hr) with free access to standard food and tap water. On the test day, rats were transferred to the recording laboratory in their home cages and allowed to adapt for approximately 1 hr prior to anesthesia.

Experimental Procedure

The baseline electroencephalogram (EEG) was recorded for 30 min following a saline injection in each rat anesthetized with isoflurane (1.5–1.8 vol% end tidal concentration). Animals ($n = 10$) received subsequently one intraperitoneal (i.p.) dose of 50 mg/kg ibogaine hydrochloride (IBO; Sigma Chemical Co., St. Louis, MO) dissolved in deionized water. The remaining rats ($n = 6$) were anesthetized and injected with saline only to serve as independent controls for the neurochemical part of the study. The EEG was recorded in IBO-treated as well as independent control rats for 2 hr. All rats were immediately sacrificed and the brains dissected into frontal cortex (FC), caudate nucleus (CN), and brain stem (BS). Concentrations of dopamine (DA), serotonin (5-HT), and their metabolites were determined by high-performance liquid chromatography with electrochemical detection (HPLC-ECD) as previously described.¹

Electrophysiological Recording and Analysis

During the procedure, animals were placed on a nonelectric isothermal pad (Braintree Scientific, Inc., Braintree, MA) in a Faraday cage to maintain body temperature and shield from external electrostatic interference. A pair of E2 subdermal platinum needle electrodes (Grass Instrument Company, Quincy, MA) were positioned near the left side of the cranium at the level of the somatosensory cortex. The indifferent electrode was located on the right flank. The position of the cranial electrodes was verified after sacrifice of the animals. The heart rate (HR) was continuously monitored via EKG E2 subdermal electrodes attached to either side of the body across the heart. The EEG signals were monitored via an oscilloscope (BK Precision 2120, Taiwan). Additionally, the EEG was preamplified by a BioAmp 100 differential amplifier ($\times 100$) and amplified subsequently by a CyberAmp 380 signal conditioner (Axon Instruments, Inc.) to pass frequencies between 1 and 40 Hz (FIG. 1).

The “Experimenter’s WorkBench” and “Common Processing” computer software packages (DataWave Technologies, Longmont, CO) were used to perform data acqui-

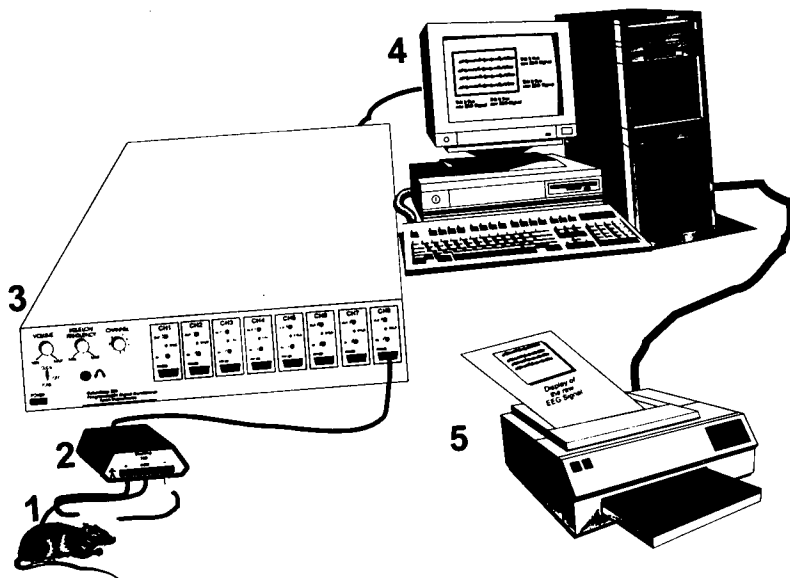


FIGURE 1. Schematic view of the EEG data acquisition/analysis system. (1) Platinum subdermal EEG electrodes installed on a cranium. (2) BioAmp 100 isolated differential amplifier, signal amplification $\times 100$. (3) CyberAmp 380 signal conditioner, frequency range 1–40 Hz. (4) Software packages (DataWave Technology) installed on EPS Technologies 486/66 MHz computer: Experimenter's Work Bench for data acquisition; Common Processing for data analysis. (5) Data display.

sition and processing. The analysis of power spectra 1–4 Hz (delta), 4–8 Hz (theta), 8–13 Hz (alpha), and 13–32 Hz (beta) was performed using fast Fourier transformations. In-house software was used to average digital signals within each frequency band over 30-sec time intervals. The averaged signals were subsequently formatted into blocks compatible with the statistical data analysis program (SAS).

Statistical Analysis

EEG Power Spectra

For individual animals, the average EEG power obtained during the baseline recording period was calculated for each frequency band and for total power. Similarly, average EEG power for each band was computed for 15-min intervals following the baseline period. Relative power for each animal and band during the postbaseline 15-min period was calculated by dividing its average by the baseline average for an individual animal and band. A log-transform was then applied to these data to improve the distribution characteristics for the statistical analysis. At this point, all animals were measured using the same scale, and all transformations were performed within each animal. For each 15-min interval and each frequency band, the logarithm of the relative power was averaged between animals, and the resulting mean was compared to 1.0 (100%) using the Student's *t* test. The resulting *p*-value gives the statistical significance of any

change in power within that frequency band as a result of the treatment. For this study, $p < 0.05$ was considered statistically significant.

Neurochemical Data

A one-way analysis of variance with Bonferroni's method of multiple comparisons was applied to detect differences in monoamine concentrations between control and IBO-treated rats. The level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Injections of saline were not associated with any marked changes in baseline EEG power spectra or the HR. Compared with baseline, a decrease in the HR immediately after ibogaine injection was observed (FIG. 2). The HR remained at the significantly lower than baseline level throughout the recording (348 ± 10 baseline vs 272 ± 12 postinjection time, mean $\pm$ SEM, $p < 0.05$). In contrast, no decrease in HR was observed in rats when saline, deionized water, or bovine serum albumin (BSA) were injected i.p. (data not shown), suggesting that a decline in the HR after IBO was an autonomic response to the IBO exposure. A decline in pulse rate concomitant with a rise in blood pressure was also reported in human patients 1–2.5 hr after oral administration of therapeutic doses of ibogaine.⁷

Compared with the baseline, a power decline in all four EEG frequency bands was observed during the first 30 min after IBO treatment (FIG. 3). The mean total power (the relative power of the whole frequency range 1–32 Hz) was reduced to 60–72% during the first 60 min following IBO exposure. While the power of the delta band remained significantly below baseline throughout the experiment, the power of the alpha, beta, and theta bands returned to the baseline level within 60 min of the IBO treatment (TABLE 1).

Compared with the independent control in the present study, total DA tissue level (extra and intracellular DA concentration) measured at the end of recording had a tendency to decline in FC of IBO-treated rats. The concentration of DA decreased and DA turnover increased significantly in CN (TABLE 2). Concomitantly, a trend toward an in-

TABLE 1. *p*-Values of the Comparisons *t* Tests between Baseline (100%) and Relative Power within Delta, Theta, Alpha, and Beta EEG Frequency Bands in 15-Min Time Intervals following IBO Treatment

Time after IBO Treatment	Delta <i>p</i> -Value	Frequency		Bands	
		Theta <i>p</i> -Value	Alpha <i>p</i> -Value	Beta <i>p</i> -Value	
1–15 min	0.02*	0.02*	0.009*	0.0006*	
16–30 min	0.02*	0.03*	0.02*	0.002*	
31–45 min	0.002*	0.06	0.2	0.04*	
46–60 min	0.001*	0.03*	0.3	0.1	
61–75 min	0.006*	0.4	0.7	0.8	
76–90 min	0.007*	0.6	0.9	0.9	

Note: $n = 10$.

*denotes statistical significance at $p < 0.05$.

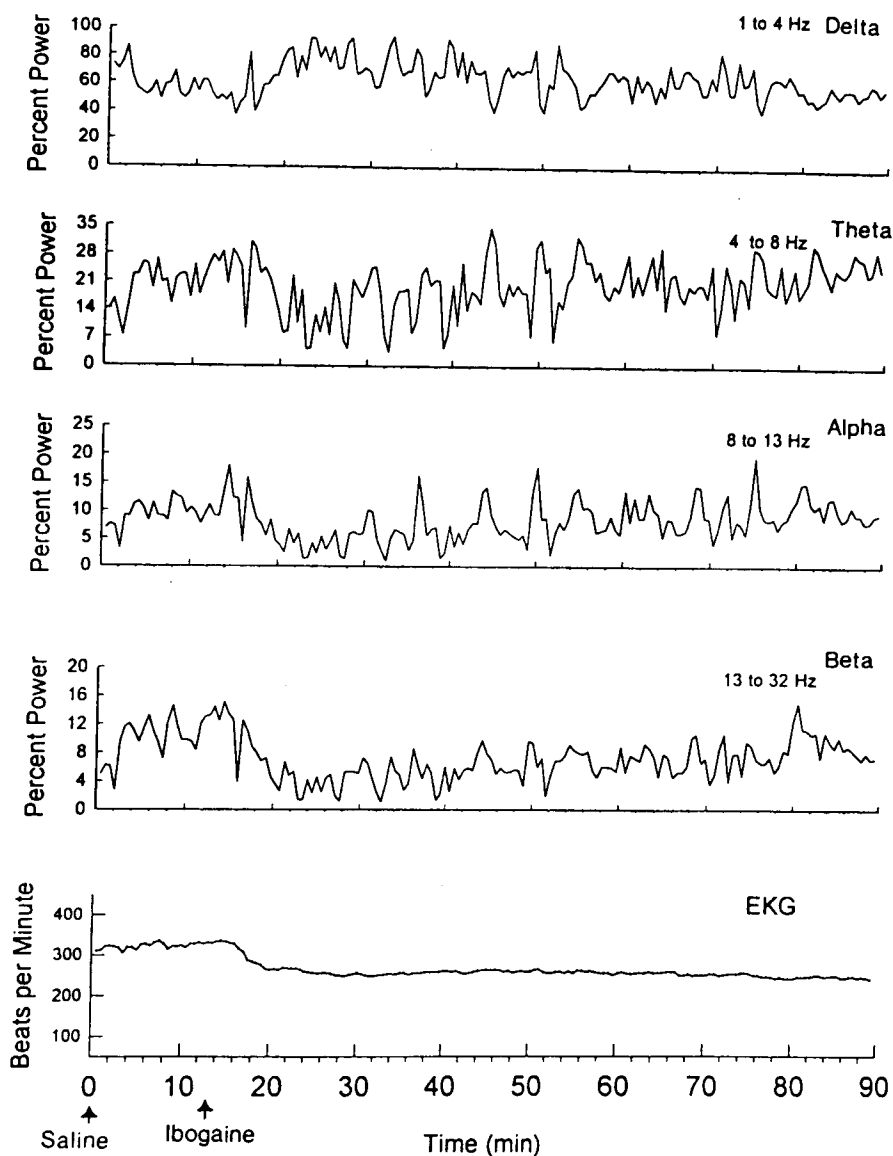


FIGURE 2. The response of an individual rat (# 55) to intraperitoneal ibogaine administration (50 mg/kg). EEG: percent power, normalized values of power spectra (percent of total power) in delta (1–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), and beta (13–32 Hz) frequency bands. EKG: heart rate (beats per minute).

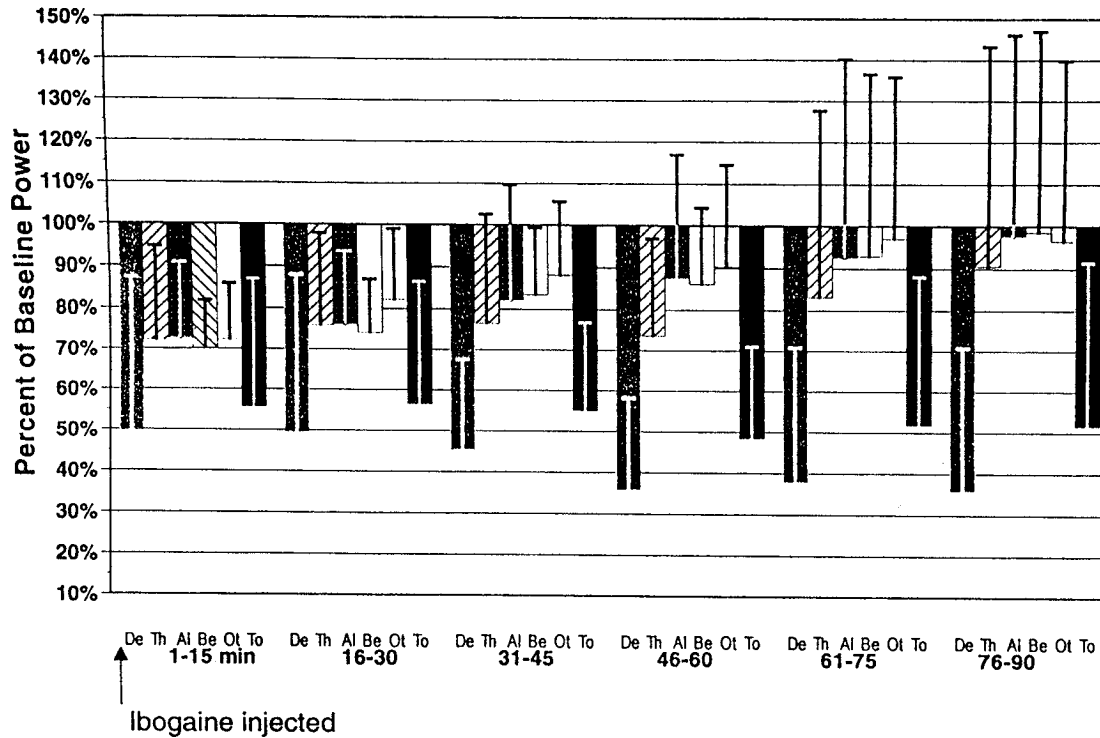


FIGURE 3. Time course of the effects produced by intraperitoneal ibogaine administration (50 mg/kg) on cortical EEG power spectra. Power values calculated as percent of the 30-min baseline power assumed 100% in each band. De, delta (1–4 Hz); Th, theta (4–8 Hz); Al, alpha (8–13 Hz); Be, beta (13–32 Hz); Ot, other (32–40 Hz); To, total power. $n = 10$; 95% confidence interval on mean of log-transformed data. Notice: If errorbar crosses 100% line then difference is not significant at $p < 0.05$.

TABLE 2. Concentrations of Dopamine and Dopamine Metabolites DOPAC and HVA (ng/100 mg of Wet Weight of Tissue) in Various Rat Brain Regions after Ibogaine Treatment (50 mg/kg)

Brain Region		Dopamine	DOPAC	HVA	DOPAC+HVA DA
FC	control	47.6 (12.8)	26.8 (2.5)	19.9 (1.1)	1.0 (0.4)
	IBO	35.8 (1.7)	25.1 (2.4)	29.6 (4.7)	1.5 (0.3)
CN	control	1093 (192)	171.2 (40.7)	158.5 (45.8)	0.3 (0.03)
	IBO	706 (112)*	194.5 (33.3)	226.8 (38.7)	0.6 (0.06)*
BS	control	26.8 (0.87)	17.43 (0.58)	6.17 (2.88)	0.9 (0.1)
	IBO	28.2 (2.20)	19.20 (0.77)	7.23 (1.93)	0.9 (0.2)

Note: DOPAC, dihydroxyphenylacetic acid; HVA, homovanillic acid. DOPAC + HVA denotes dopamine turnover. Mean $\pm$ SEM; $n = 6$.

* $p < 0.05$ significantly different from control.

from 54.9 ± 8.63 in control rats to 86.2 ± 16.14 ng/100 mg of wet tissue in IBO-treated rats.

A depletion of DA level and/or an increase of 5-HT after IBO treatment was reported previously by our group as well as other investigators.^{1,8,9} The extracellular DA release observed in the *in vivo* studies using microdialysis was suggested to account, at least in part, for the decline of the total DA concentration in the cortex and striatum following IBO treatment.⁸ In the present study, the deactivation of all four power bands corresponded with a decline in total DA observed during the first 30 min after IBO treatment in a previous time course study.¹ The selective power decrease in delta and alpha EEG frequency bands of the cortical EEG may suggest activation of dopamine receptors as suggested by other investigators.³ A decrease in total DA observed in this study might be related to the IBO-stimulated extracellular DA release.

Studies in rats have documented effects of IBO and its metabolite, noribogaine, on dopaminergic and serotonergic systems.^{6,8,9} The interaction of ibogaine and its metabolites with dopamine, serotonin and/or NMDA receptor sites were hypothesized to be responsible for the therapeutic effect of IBO.^{6,9,13} In the present study, both peripheral, cardiovascular serotonergic-like (bradycardia) and central, EEG dopaminergic-like (delta and alpha band deactivation) response appears to follow acute IBO exposure.

In summary, intraperitoneal administration of IBO resulted in a prolonged power

TABLE 3. Concentrations of Serotonin and the Serotonin Metabolite 5-HIAA (ng/100 mg of Wet Weight of Tissue) in Various Rat Brain Regions after Ibogaine Treatment (50 mg/kg). $n=6$; mean ($\pm$ SEM).

Brain Region		5-HT	5-HIAA
FC	control	54.9 (8.63)	18.2 (3.9)
	IBO	86.2 (16.1)	25.4 (6.8)
CN	control	71.7 (7.98)	22.5 (2.7)
	IBO	50.6 (6.53)	24.1 (0.6)
HIP	control	65.7 (8.21)	22.1 (1.5)
	IBO	49.3 (5.52)	27.4 (2.2)
BS	control	72.37 (5.06)	51.3 (2.9)
	IBO	73.78 (6.75)	55.5 (9.5)

Note: 5-HIAA, 5-hydroxyindoleacetic acid. Means $\pm$ SEM; $n = 6$.

decline in the EEG delta band, and heart rate throughout the 90-min EEG recording. These alterations were accompanied with a decrease in the total DA level observed 90 min after the injection. These effects suggest mainly serotonergic and dopaminergic response to IBO exposure.

CONCLUSION

With regard to neurochemical and electrophysiological endpoints examined *in vivo*, it appears that the cardiovascular and brain responses to acute IBO exposure observed in this study were associated primarily with the serotonergic and dopaminergic systems.

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